

F A C S I M I L E	
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Re: U.S. Patent Application No. 10/694,847 Your Ref: 44 534 K (Parent Reference) Our Ref: KOENIG-0002-D01	
Total No. of Pages: 2; if you do not receive all pages, please call (703) 243-6333	

Dear Examiner Rooke:

Further to our conversation, please amend claims 23 and 29 by removing the term "or prevented" from each of these claims.

The term written out for PTCA/PTA from claim 24 and elsewhere in the application is Percutaneous Transluminal Coronary Angioplasty / Percutaneous Transluminal Angioplasty. Please make changes where necessary.

If you have any questions, please do not hesitate to contact us.

Very truly yours,

  
Csaba Henter

K:\koenig\2D1\fax to Examiner.dot

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**Amendments to the Specification**

**Please insert the paragraph below the title and prior to the first paragraph of the specification.**

This application is a divisional of U.S. Application No. 09/445,214 filed 5/3/00 which is a 371 of PCT/EP98/03356 filed on 6/5/98.

*, now abandoned,*



Agnes Poole  
06/09/2006

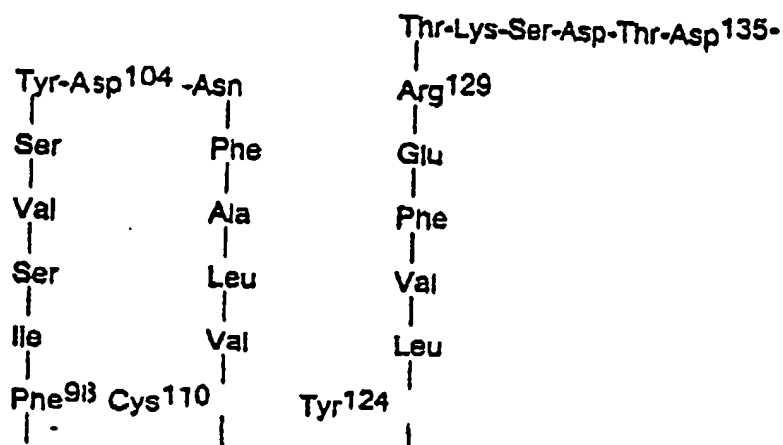
### Example 1

Determination of the

1. ~~Determination of the~~ Crystal Structure of a Complex that Consists of Thrombin and Triabin

Purified triabin and thrombin were added together in 20 mmol of sodium acetate, 25 mmol of sodium chloride, pH 5.5. Crystals of the complex of triabin and thrombin formed in a hanging drop, which contained 50 mmol of sodium acetate, pH 4.7, 100 mmol of ammonium sulfate, 0.01% sodium nitrite and 8% PEG 4000. The structure of the crystals was determined by means of x-ray analysis. The amino acids, which form interactions with thrombin, were determined from these structural data. It has been shown that these amino acids are found in areas that form a  $\beta$ -folded-sheet structure.

The partial sequences of triabin, which bind to thrombin, read:



area of amino acids 98-103 (named chain 1), amino acids 105-110 (chain 2) and amino acids 124-135 (chain 3) must be sterically stabilized. This could be achieved by different modifications of the original areas according to the following batches.

## 2. Stabilization of the Peptides

### A. Stabilization of Chains 1 and 2

The stabilization was carried out either by

1. Exchange of the amino acids phenylalanine in triabin in 98-position and/or cysteine in 110-position for amino acids, which allow a linkage via the side chain (e.g., Lys-Asp, Cys-Cys, ornithine-Glu)

or by

2. Exchange of Cys110 for Asp and linkage of Phe98 and this Asp by a " $\beta$ -turn mimetic agent." The structures of  $\beta$ -turn mimetic agents and their use are described in detail in U. Egner et al. (1997) Pesticide Science, in Press.

### → B. ~~The~~ Stabilization of Chains 2 and 3

The stabilization was carried out either by

1. Exchange of the L-Tyr in 124-position for a D-Tyr (configuration isomer) and linkage of amino acids in 110-position (in the triabin Cys) with that in 124-position (in the triabin Tyr) by a peptide bond

or by

2. Exchange of the L-Tyr in 124-position for a D-Tyr and linkage of the amino acid in 110-position (in the triabin Cys)

*Agnes Padua  
06/05/06*

Agnes Poole  
66/09/06 →

### *the Peptide*

#### 1. ~~Synthesis of the Peptide~~

The synthesis was carried out according to standard methods in a peptide-synthesis machine of Applied Biosystems.

Sequence: Lys-Ile-Ser-Val-Ser-Tyr-Asp-Asn-Phe-Ala-Leu-Val-Asp-D-Tyr-Leu-Val-Phe-Glu-Arg-Thr-Lys-Ser-Asp-Thr-Asp

#### 2. Cleavage of the Dde and O-Al1 Groups

In addition, the resin was washed twice with DMSO/DCM (1:1) and allowed to steep in this solution for 30 minutes. Then, palladium (0.1 mol/mol of peptide) and DPPF (0.1 mol/mol of peptide) and acetic acid (10-fold excess) were added.  $\text{Sn}(\text{Bu})_3\text{H}$  was added in 5 portions (a total of 5-fold excess) within 10 minutes. The mixture was stirred for 20 minutes, then suctioned off and washed with DMSO/DCM and DCM.

#### 3. Cyclization

The resin was pre-steeped in DMF for 30 minutes, then DIPEA (8-fold excess), TBTU (2-fold excess) and HOBT (2-fold) were added and stirred overnight. The resin was suctioned off and washed with DMF and ether.

#### 4. Cleavage of Resin and Cleavage of the Residual Protective Groups

Phenol, ethyldithiol, thioanisole,  $\text{H}_2\text{O}$  and TFA were added to the resin, and the reaction mixture was stirred for 4 hours at  $37^\circ\text{C}$ . The peptide was precipitated with t-butylether, centrifuged off and dried under nitrogen.

## Sample Application 3

*the Fibrinogen cleavage*  
Measurement of ~~th Fibrinogen cleavage~~

Microtiter plates were coated first with bovine serum albumin. Then, 100  $\mu$ l of triabin solution (0.1-10  $\mu$ mol/l 10 mmol of Na H<sub>2</sub>PO<sub>4</sub>, pH 7.4) was added to the microtiter plate, and 100  $\mu$ l of reaction buffer (20 mmol of HEPES, 0.15 M of NaCl, pH 7.4), 20  $\mu$ l of CaCl<sub>2</sub> solution (20 mmol of CaCl<sub>2</sub> in H<sub>2</sub>O) and 20  $\mu$ l of thrombin solution (0.012 IU) were pipetted into it. After an incubation at 37°C for 2 minutes, 100  $\mu$ l of fibrinogen (10 mg in 2 ml of reaction buffer) was added and incubated for 40 minutes at 37°C. Then, the extinction was measured at 405 nm. As a control value (100% thrombin activity), 100  $\mu$ l of reaction buffer was used in a batch instead of the triabin solution. The measured values with triabin were related to these values and expressed as % of inhibition.